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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

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TITLE OF THE INVENTION (500 characters max)

VLA-4 ANTAGONISTS

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Respectfully submitted,

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Date 10/01/2004

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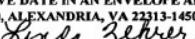
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TITLE OF THE INVENTION

VLA-4 ANTAGONISTS

BACKGROUND OF THE INVENTION

5 VLA-4 ("very late antigen-4"; CD49d/CD29; or $\alpha_4\beta_1$) is an integrin expressed on all leukocytes, except platelets and mature neutrophils, including dendritic cells and macrophage-like cells and is a key mediator of the cell-cell and cell-matrix interactions of these cell types. The ligands for VLA-4 include vascular cell adhesion molecule-1 (VCAM-1), the CS-1 domain of fibronectin (FN), and the matrix protein, osteopontin. Neutralizing anti- α_4 antibodies or blocking peptides that inhibit the
10 interaction between VLA-4 and its ligands have been shown to be efficacious both prophylactically and therapeutically in several animal models of disease including asthma, multiple sclerosis, inflammatory bowel disease, and rheumatoid arthritis.

The humanized monoclonal antibody against α_4 , natalizumab (Antegren®, Elan/Biogen), has demonstrated efficacy in the treatment of multiple sclerosis (D. H. Miller et al., *New England Journal of Medicine*, **348**, 15 (2003)) and Crohn's disease (S. Ghosh et al. *New England Journal of Medicine*, **348**, 23 (2003)). There are also several VLA-4 antagonists in early clinical trials for treatment of asthma, arthritis, multiple sclerosis, and Crohn's disease.

In the early clinical trials with natalizumab, lymphocytosis (a surrogate marker for blockade of VLA-4 function) and > 80% receptor occupancy were observed. A small molecule VLA-4
20 antagonist was reported to demonstrate functional activity in the rat experimental autoimmune encephalomyelitis (EAE) assay, an animal model of multiple sclerosis following subcutaneous administration (D. R. Leone et al., *J. Pharmacol. Exper. Therap.*, **305**, 1150 (2003)). This compound was shown to induce lymphocytosis, and to have a slow dissociation rate (off-rate) resulting in significant and sustained receptor occupancy on VLA-4-bearing cells. There was a positive correlation between receptor
25 occupancy, lymphocytosis, and efficacy in the EAE model described in this manuscript.

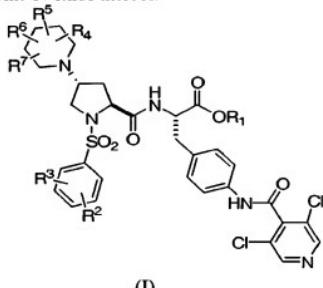
A series of isonicotinoyl-L-aminophenylalanine derivatives shown to possess slow dissociation (off-rate) from VLA-4 on Jurkat cells were reported in G. Doherty et al., *Bioorganic & Medicinal Chemistry Letters*, **13**, 1891 (2003). However, the compound that was further characterized demonstrated very poor pharmacokinetic properties such as low oral bioavailability, moderate to high
30 plasma clearance and a short half-life rendering it unsuitable for oral administration. Compounds of the present invention are potent antagonists of VLA-4 capable of achieving and maintaining receptor occupancy for a time sufficient to allow for oral administration.

SUMMARY OF THE INVENTION

Substituted N-[N-benzenesulfonyl-prolyl]-phenylalanine derivatives of the present invention are antagonists of the VLA-4 integrin and are useful in the treatment, prevention and suppression of diseases mediated by VLA-4-binding and cell adhesion and activation. Moreover, the compounds of the present invention demonstrate significant receptor occupancy of VLA-4 bearing cells after oral administration and are suitable for once-, twice-, or thrice-a-day oral administration. This invention also relates to compositions containing such compounds and methods of treatment using such compounds.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a compound of formula I, or a pharmaceutically acceptable salt thereof, or pyridine-N-oxide thereof:



wherein

R¹ is selected from (1) hydrogen, (2) C₁-10alkyl, (3) -(C₁-10alkyl)-aryl, (4) -(C₁-10alkyl)-O-C₁-10alkyl, (5) -(C₁-10alkyl)-OC(O)-C₁-10alkyl, (6) -(C₁-10alkyl)-OC(O)-aryl, (7) -(C₁-10alkyl)-OC(O)O-C₁-10alkyl, and (8) -(C₁-10alkyl)-N+(C₁-3alkyl)3;

R² and R³ are independently selected from H, -SO₂-C₁-3alkyl, CN, CF₃, OCF₃, and halogen;

R⁴, R⁵, R⁶ and R⁷ are independently selected from hydrogen and C₁-10alkyl; or

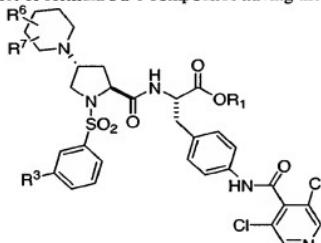
two of R⁴, R⁵, R⁶ and R⁷ together with the carbon atom(s) to which they are attached complete a 3-8 membered carbocyclic ring.

In one subset of formula I are compounds wherein R¹ is hydrogen, C₁-4alkyl, -(C₁-4alkyl)OC₁-4alkyl, or -(C₁-4alkyl)N⁺(C₁-3alkyl)3. In one embodiment thereof R¹ is selected from hydrogen, methyl, ethyl, 2-methoxyethyl, and 2-(trimethylaminium)ethyl. In a second embodiment thereof, R¹ is hydrogen, methyl or ethyl.

In a second subset of formula I are compounds wherein R² is hydrogen and R³ is selected from -SO₂-C₁₋₃alkyl, CN and halogen. In one embodiment thereof R² is hydrogen and R³ is selected from methanesulfonyl, CN, and halogen. In a second embodiment thereof R² is hydrogen and R³ is CN.

5 In a third subset of formula I are compounds wherein R⁴ and R⁵ are each hydrogen, and R⁶ and R⁷ are independently hydrogen or C₁₋₄alkyl. In one embodiment thereof R⁴, R⁵, R⁶ and R⁷ are each hydrogen. In a second embodiment therof R⁴, R⁵ and R⁶ are each hydrogen, and R⁷ is C₁₋₄alkyl. In a third embodiment thereof R⁴ and R⁵ are each hydrogen, and R⁶ and R⁷ are independently C₁₋₄alkyl

In a fourth subset of formula I are compounds having the formula Ia:



10

Ia

or a pharmaceutically acceptable salt thereof, or pyridine N-oxide thereof, wherein R¹ is selected from hydrogen, C₁₋₁₀alkyl, -(C₁₋₄alkyl)-aryl, -(C₁₋₄alkyl)-O-C₁₋₄alkyl, and -(C₁₋₄alkyl)-N⁺(C₁₋₃alkyl)₃;

15 R³ is CN, halogen or -SO₂-C₁₋₃alkyl; and

R⁶ and R⁷ are independently hydrogen or C₁₋₄alkyl.

In one embodiment of compounds of formula Ia R³ is CN. In a second embodiment R⁶ and R⁷ are each hydrogen.

Representative examples of compounds of formula I include, but are not limited to,

- 20 (4*R*)-1-[(3-cyanophenyl)sulfonyl]-4-(piperidin-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)amino]-L-phenylalanine;
- (4*R*)-1-[(3-cyanophenyl)sulfonyl]-4-(2-methylpiperidin-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalanine;
- (4*R*)-1-[(3-cyanophenyl)sulfonyl]-4-(3-methylpiperidin-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalanine;
- 25

- (4*R*)-1-[(3-cyanophenyl)sulfonyl]-4-(4-methylpiperidin-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalanine;
- (4*R*)-1-[(3-cyanophenyl)sulfonyl]-4-(3,5-dimethylpiperidin-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalanine;
- 5 (4*R*)-1-[(3-cyanophenyl)sulfonyl]-4-(3,3-dimethylpiperidin-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalanine;
- (4*R*)-1-[(3-cyanophenyl)sulfonyl]-4-(4,4-dimethylpiperidin-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalanine;
- (4*R*)-1-[(3-cyanophenyl)sulfonyl]-4-(octahydroquinolin-1(2*H*)-yl)-L-prolyl-4-[(3,5-dichloro-10 isonicotinoyl)amino]-L-phenylalanine;
- (4*R*)-1-[(3-cyanophenyl)sulfonyl]-4-(octahydroisoquinolin-2(1*H*)-yl)-L-prolyl-4-[(3,5-dichloro-isonicotinoyl)amino]-L-phenylalanine;
- (4*R*)-4-(2-azabicyclo[2.2.2]oct-2-yl)-1-[(3-cyanophenyl)sulfonyl]-L-prolyl-4-[(3,5-dichloro-isonicotinoyl)amino]-L-phenylalanine;
- 15 Ethyl (4*R*)-1-[(3-bromophenyl)sulfonyl]-4-piperidin-1-yl-L-prolyl-4-[(3,5-dichloroisonicotinoyl)amino]-L-phenylalaninate;
and pharmaceutically acceptable salts thereof.

In another aspect the present invention provides a method for the prevention or treatment of diseases, disorders, conditions or symptoms mediated by cell adhesion in a mammal which comprises 20 administering to said mammal an effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof. This aspect includes the use of a compound of formula I or a pharmaceutically acceptable salt thereof in the manufacture of a medicament for the treatment of diseases, disorders, conditions or symptoms mediated by cell adhesion in a mammal. In one embodiment said disease or disorder is selected from asthma, allergic rhinitis, multiple sclerosis, atherosclerosis, inflammatory bowel 25 disease, rheumatoid arthritis, organ transplantation, acute leukemia, and sickle cell anemia.

In another aspect the present invention provides a method for preventing the action of VLA-4 in a mammal which comprises administering to said mammal a therapeutically effective amount of a compound of formula I or a pharmaceutically acceptable salt thereof. This aspect includes the use of a compound of formula I in the manufacture of a medicament for preventing the action of VLA-4 in a 30 mammal.

Another aspect of the present invention provides a pharmaceutical composition which comprises a compound of formula I or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

"Alkyl", as well as other groups having the prefix "alk", such as alkoxy, alkanoyl, means carbon chains which may be linear or branched or combinations thereof. Examples of alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, sec- and tert-butyl, pentyl, hexyl, heptyl, octyl, nonyl, and the like.

- 5 "Aryl" means mono- or bicyclic aromatic rings containing only carbon atoms. The term also includes aryl group fused to a monocyclic cycloalkyl or monocyclic heterocyclyl group in which the point of attachment is on the aromatic portion. Examples of aryl include phenyl, naphthyl, indanyl, indenyl, tetrahydronaphthyl, 2,3-dihydrobenzofuranyl, dihydrobenzopyranyl, 1,4-benzodioxanyl, and the like.
- 10 "Halogen" includes fluorine, chlorine, bromine and iodine.

Optical Isomers - Diastereomers - Geometric Isomers - Tautomers

Compounds of Formula I contain one or more asymmetric centers and can thus occur as racemates and racemic mixtures, single enantiomers, diastereomeric mixtures and individual 15 diastereomers. The present invention is meant to comprehend all such isomeric forms of the compounds of Formula I.

Some of the compounds described herein contain olefinic double bonds, and unless specified otherwise, are meant to include both E and Z geometric isomers.

Some of the compounds described herein may exist with different points of attachment 20 of hydrogen, referred to as tautomers. Such an example may be a ketone and its enol form known as keto-enol tautomers. The individual tautomers as well as mixture thereof are encompassed with compounds of Formula I.

Compounds of the Formula I may be separated into diastereoisomeric pairs of enantiomers by, for example, fractional crystallization from a suitable solvent, for example MeOH or 25 EtOAc or a mixture thereof. The pair of enantiomers thus obtained may be separated into individual stereoisomers by conventional means, for example by the use of an optically active amine as a resolving agent or on a chiral HPLC column.

Alternatively, any enantiomer of a compound of the general Formula I or Ia may be obtained by stereospecific synthesis using optically pure starting materials or reagents of known 30 configuration.

Salts

The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic or organic bases and inorganic

or organic acids. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, zinc, and the like. Particularly preferred are the ammonium, calcium, magnesium, potassium, and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, 5 and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, and basic ion exchange resins, such as arginine, betaine, caffeine, choline, N,N'-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethyl-morpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydрабамине, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, 10 theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and the like.

When the compound of the present invention is basic, salts may be prepared from pharmaceutically acceptable non-toxic acids, including inorganic and organic acids. Such acids include acetic, benzenesulfonic, benzoic, camphorsulfonic, citric, ethanesulfonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, 15 pamoic, pantothenic, phosphoric, succinic, sulfuric, tartaric, p-toluenesulfonic acid, and the like. Particularly preferred are citric, hydrobromic, hydrochloric, maleic, phosphoric, sulfuric, and tartaric acids.

It will be understood that, as used herein, references to the compounds of Formula I are meant to also include the pharmaceutically acceptable salts.

20

Utilities

The ability of the compounds of Formula I to antagonize the actions of VLA-4 integrin makes them useful for preventing or reversing the symptoms, disorders or diseases induced by the binding of VLA-4 to its various ligands. Thus, these antagonists will inhibit cell adhesion processes 25 including cell activation, migration, proliferation and differentiation. Accordingly, another aspect of the present invention provides a method for the treatment (including prevention, alleviation, amelioration or suppression) of diseases or disorders or symptoms mediated by VLA-4 binding and cell adhesion and activation, which comprises administering to a mammal an effective amount of a compound of Formula I. Such diseases, disorders, conditions or symptoms are, for example (1) multiple sclerosis, (2) asthma, (3) 30 allergic rhinitis, (4) allergic conjunctivitis, (5) inflammatory lung diseases, (6) rheumatoid arthritis, (7) septic arthritis, (8) type I diabetes, (9) organ transplantation rejection, (10) restenosis, (11) autologous bone marrow transplantation, (12) inflammatory sequelae of viral infections, (13) myocarditis, (14) inflammatory bowel disease including ulcerative colitis and Crohn's disease, (15) certain types of toxic and immune-based nephritis, (16) contact dermal hypersensitivity, (17) psoriasis, (18) tumor metastasis,

(19) atherosclerosis, (20) sickle cell anemia, (21) certain acute leukemias, (22) various melanomas, carcinomas and sarcomas (including multiple myeloma); (23) acute respiratory distress syndrome; (24) uveitis; (25) circulatory shock; and (26) hepatitis.

The utilities of the present compounds in these diseases or disorders may be demonstrated in animal disease models that have been reported in the literature. The following are examples of such animal disease models: i) experimental allergic encephalomyelitis, a model of neuronal demyelination resembling multiple sclerosis (for example, see T. Yednock et al., *Nature*, 356, 63 (1993) and E. Keszthelyi et al., *Neurology*, 47, 1053 (1996)); ii) bronchial hyperresponsiveness in sheep and guinea pigs as models for the various phases of asthma (for example, see W. M. Abraham et al., *J. Clin. Invest.*, 93, 776 (1993) and A. A. Y. Milne and P. P. Piper, *Eur. J. Pharmacol.*, 282, 243 (1995)); iii) adjuvant-induced arthritis in rats as a model of inflammatory arthritis (see C. Barbadillo et al., *Arthr. Rheuma.* (Suppl.), 36 95 (1993) and D. Seiffge, *J. Rheumatol.*, 23, 12 (1996)); iv) adoptive autoimmunity diabetes in the NOD mouse (see J. L. Baron et al., *J. Clin. Invest.*, 93, 1700 (1994), A. Jakubowski et al., *J. Immunol.*, 155, 938 (1995), and X. D. Yang et al., *Diabetes*, 46, 1542 (1997)); v) cardiac allograft survival in mice as a model of organ transplantation (see M. Isobe et al., *Transplant. Proc.*, 26, 867 (1994) and S. Molossi et al., *J. Clin. Invest.*, 95, 2601 (1995)); vi) spontaneous chronic colitis in cotton-top tamarins which resembles human ulcerative colitis, a form of inflammatory bowel disease (see D. K. Podolsky et al., *J. Clin. Invest.*, 92, 372 (1993)); vii) contact hypersensitivity models as a model for skin allergic reactions (see T. A. Ferguson and T. S. Kupper, *J. Immunol.*, 150, 1172 (1993) and P. L. Chisholm et al., *Eur. J. Immunol.*, 23, 682 (1993)); viii) acute nephrotoxic nephritis (see M. S. Mulligan et al., *J. Clin. Invest.*, 91, 577 (1993)); ix) tumor metastasis (for examples, see M. Edward, *Curr. Opin. Oncol.*, 7, 185 (1995)); x) experimental autoimmune thyroiditis (see R. W. McMurray et al., *Autoimmunity*, 23, 9 (1996)); xi) ischemic tissue damage following arterial occlusion in rats (see F. Squadrato et al., *Eur. J. Pharmacol.*, 318, 153 (1996)); xii) inhibition of TH2 T-cell cytokine production including IL-4 and IL-5 by VLA-4 antibodies which would attenuate allergic responses (*J. Clinical Investigation* 100, 3083 (1997); xiii) antibodies to VLA-4 integrin mobilize long term repopulating cells and augment cytokine-induced mobilization in primates and mice (*Blood*, 90 4779-4788 (1997); xiv) sickle reticulocytes adhere to VCAM-1 (*Blood* 85 268-274 (1995) and *Blood* 88 4348-4358 (1996); xv) chemokine stromal cell derived factor 1 modulates VLA-4 integrin mediated multiple myeloma cell adhesion to CS-1/fibronectin and VCAM-1 (*Blood*, 97, 346-351 (2001))

Dose Ranges

The magnitude of prophylactic or therapeutic dose of a compound of Formula I will, of course, vary with the nature and severity of the condition to be treated, and with the particular compound

of Formula I used and its route of administration. The dose will also vary according to the age, weight and response of the individual patient. In general, the daily dose range lie within the range of from about 0.001 mg to about 100 mg per kg body weight of a mammal, preferably 0.01 mg to about 50 mg per kg, and most preferably 0.1 to 10 mg per kg, in single or divided doses. On the other hand, it may be

5 necessary to use dosages outside these limits in some cases.

For use where a composition for intravenous administration is employed, a suitable dosage range is from about 0.01 mg to about 25 mg (preferably from 0.1 mg to about 10 mg) of a compound of Formula I per kg of body weight per day.

In the case where an oral composition is employed, a suitable dosage range is, e.g. from
10 about 0.01 mg to about 100 mg of a compound of Formula I per kg of body weight per day, preferably from about 0.1 mg to about 10 mg per kg.

For use where a composition for sublingual administration is employed, a suitable dosage range is from 0.01 mg to about 25 mg (preferably from 0.1 mg to about 5 mg) of a compound of Formula I per kg of body weight per day.

15 For the treatment of asthma, a compound of Formula I may be used at a dose of from about 0.1 mg/kg to about 100 mg/kg, preferably from about 1 mg/kg to 10 mg/kg, by oral/inhalation/sublingual/etc. once, twice, three times daily, etc. The dose may be administered as a single daily dose or divided for twice or thrice daily administration.

For the treatment of multiple sclerosis, a compound of Formula I may be used at a dose
20 of from about 0.1 mg/kg to about 100 mg/kg, preferably from about 1 mg/kg to 10 mg/kg, by oral/inhalation/sublingual/etc. once, twice, three times daily, etc. The dose may be administered as a single daily dose or divided for twice or thrice daily administration.

For the treatment of inflammatory bowel disease, a compound of Formula I may be used at a dose of from about 0.1 mg/kg to about 100 mg/kg, preferably from about 1 mg/kg to 10 mg/kg, by
25 oral/inhalation/etc. once, twice, three times daily, etc. The dose may be administered as a single daily dose or divided for twice or thrice daily administration.

For the treatment of rheumatoid arthritis, a compound of Formula I may be used at a dose of from about 0.1 mg/kg to about 100 mg/kg, preferably from about 1 mg/kg to 10 mg/kg, by
30 oral/inhalation/sublingual/etc. once, twice, three times daily, etc. The dose may be administered as a single daily dose or divided for twice or thrice daily administration.

Pharmaceutical Compositions

Another aspect of the present invention provides pharmaceutical compositions which comprises a compound of Formula I and a pharmaceutically acceptable carrier. The term "composition",

as in pharmaceutical composition, is intended to encompass a product comprising the active ingredient(s), and the inert ingredient(s) (pharmaceutically acceptable excipients) that make up the carrier, as well as any product which results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, 5 or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions of the present invention encompass any composition made by admixing a compound of Formula I, additional active ingredient(s), and pharmaceutically acceptable excipients.

Any suitable route of administration may be employed for providing a mammal, especially a human with an effective dosage of a compound of the present invention. For example, oral, 10 sublingual, rectal, topical, parenteral, ocular, pulmonary, nasal, and the like may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, creams, ointments, aerosols, and the like.

The pharmaceutical compositions of the present invention comprise a compound of 15 Formula I as an active ingredient or a pharmaceutically acceptable salt thereof, and may also contain a pharmaceutically acceptable carrier and optionally other therapeutic ingredients. The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases or acids including inorganic bases or acids and organic bases or acids.

The compositions include compositions suitable for oral, sublingual, rectal, topical, parenteral (including subcutaneous, intramuscular, and intravenous), ocular (ophthalmic), pulmonary 20 (aerosol inhalation), or nasal administration, although the most suitable route in any given case will depend on the nature and severity of the conditions being treated and on the nature of the active ingredient. They may be conveniently presented in unit dosage form and prepared by any of the methods well-known in the art of pharmacy.

For administration by inhalation, the compounds of the present invention are 25 conveniently delivered in the form of an aerosol spray presentation from pressurized packs or nebulizers. The compounds may also be delivered as powders which may be formulated and the powder composition may be inhaled with the aid of an insufflation powder inhaler device. The preferred delivery systems for inhalation are metered dose inhalation (MDI) aerosol, which may be formulated as a suspension or solution of a compound of Formula I in suitable propellants, such as fluorocarbons or hydrocarbons and 30 dry powder inhalation (DPI) aerosol, which may be formulated as a dry powder of a compound of Formula I with or without additional excipients.

Suitable topical formulations of a compound of formula I include transdermal devices, aerosols, creams, ointments, lotions, dusting powders, and the like.

In practical use, the compounds of Formula I can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of preparation desired for administration, e.g., oral or parenteral (including intravenous).

- 5 In preparing the compositions for oral dosage form, any of the usual pharmaceutical media may be employed, such as, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents and the like in the case of oral liquid preparations, such as, for example, suspensions, elixirs and solutions; or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents and the like in the case of oral solid preparations such as, for example, powders, capsules and tablets,
- 10 with the solid oral preparations being preferred over the liquid preparations. Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form in which case solid pharmaceutical carriers are obviously employed. If desired, tablets may be coated by standard aqueous or nonaqueous techniques.

15 In addition to the common dosage forms set out above, the compounds of Formula I may also be administered by controlled release means and/or delivery devices such as those described in U.S. Patent Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; 3,630,200 and 4,008,719.

20 Pharmaceutical compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient, as a powder or granules or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an oil-in-water emulsion or a water-in-oil liquid emulsion. Such compositions may be prepared by any of the methods of pharmacy but all methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the 25 product into the desired presentation. For example, a tablet may be prepared by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine, the active ingredient in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine, a mixture of the powdered compound moistened with an inert 30 liquid diluent. Desirably, each tablet contains from about 1 mg to about 500 mg of the active ingredient and each cachet or capsule contains from about 1 to about 500 mg of the active ingredient.

The following are examples of representative pharmaceutical dosage forms for the compounds of Formula I:

Inj. Suspension (I.M.)	mg/mL	Tablet	mg/tab.	Capsule	mg/cap.
Cmpd of Formula I	10	Cmpd of Formula I	25	Cmpd of Formula I	25
Methylcellulose 5.0		Microcryst. Cellulose	415	Lactose Powder	573.5
Tween 80	0.5	Povidone	14.0	Magnesium Stearate	1.5
Benzyl alcohol	9.0	Pregelatinized Starch	43.5		600
Benzalkonium chloride	1.0	Magnesium Stearate	2.5		
Water for injection to a total volume of 1 mL			500		

5 Aerosol Per canister

Compound of Formula I 24 mg
 Lecithin, NF Liq. Conc. 1.2 mg
 Trichlorofluoromethane, NF 4.025 g
 Dichlorodifluoromethane, NF 12.15 g

10

Combination Therapy

Compounds of Formula I may be used in combination with other drugs that are used in the treatment/prevention/suppression or amelioration of the diseases or conditions for which compounds of Formula I are useful. Such other drugs may be administered, by a route and in an amount commonly used therefor, contemporaneously or sequentially with a compound of Formula I. When a compound of Formula I is used contemporaneously with one or more other drugs, a pharmaceutical composition containing such other drugs in addition to the compound of Formula I is preferred. Accordingly, the pharmaceutical compositions of the present invention include those that also contain one or more other active ingredients, in addition to a compound of Formula I. Examples of other active ingredients that 15 may be combined with a compound of Formula I, either administered separately or in the same pharmaceutical compositions, include, but are not limited to: (a) other VLA-4 antagonists such as those described in US 5,510,332, WO97/03094, WO97/02289, WO96/40781, WO96/22966, WO96/20216, WO96/01644, WO96/06108, WO95/15973 and WO96/31206, as well as natalizumab; (b) steroids such as beclomethasone, methylprednisolone, betamethasone, prednisone, dexamethasone, and 20 hydrocortisone; (c) immunosuppressants such as cyclosporin, tacrolimus, rapamycin and other FK-506 type immunosuppressants; (d) antihistamines (H1-histamine antagonists) such as bromopheniramine,

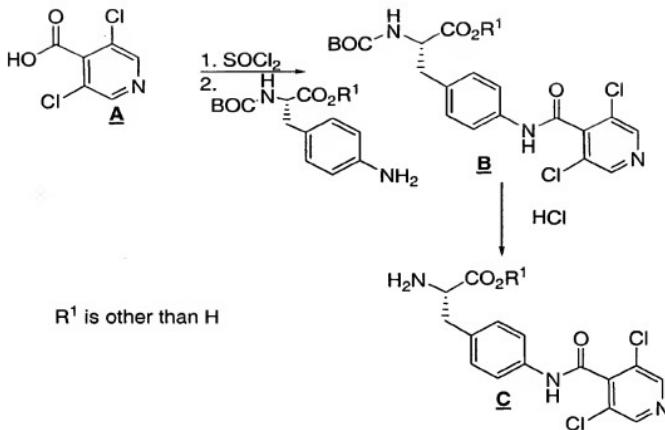
chlorpheniramine, dexchlorpheniramine, triprolidine, clemastine, diphenhydramine, diphenylpyraline, tripelennamine, hydroxyzine, methdilazine, promethazine, trimeprazine, azatadine, cyroheptadine, antazoline, pheniramine pyrilamine, astemizole, terfenadine, loratadine, cetirizine, fexofenadine, descarboethoxyloratadine, and the like; (e) non-steroidal anti-asthmatics such as β 2-agonists (terbutaline, 5 metaproterenol, fenoterol, isoproterenol, albuterol, bitolterol, salmeterol and pirbuterol), theophylline, cromolyn sodium, atropine, ipratropium bromide, leukotriene antagonists (zafirlukast, montelukast, pranlukast, 10 irlukast, pobilukast, SKB-106,203), leukotriene biosynthesis inhibitors (zileuton, BAY-1005); (f) non-steroidal antiinflammatory agents (NSAIDs) such as propionic acid derivatives (alminoprofen, benoxaprofen, bucloxic acid, carprofen, fensufen, fenoprofen, fluprofen, flurbiprofen, 15 ibuprofen, indoprofen, ketoprofen, miroprofen, naproxen, oxaprozin, pirprofen, pranoprofen, suprofen, tiaprofenic acid, and tioxaprofen), acetic acid derivatives (indomethacin, acemetacin, alclofenac, clidanac, diclofenac, fenclofenac, fenclozic acid, fentiazac, furofenac, ibufenac, isoxepac, oxpinac, sulindac, tiopinac, tolmetin, zidometacin, and zomepirac), fenamic acid derivatives (flufenamic acid, 20 meclofenamic acid, mefenamic acid, niflumic acid and tolfenamic acid), biphenylcarboxylic acid derivatives (diflunisal and flufenisal), oxicams (isoxicam, piroxicam, sudsoxicam and tenoxicam), salicylates (acetyl salicylic acid, sulfasalazine) and the pyrazolones (apazone, bezipiperonyl, feprazon, mofebutazone, oxyphenbutazone, phenylbutazone); (g) cyclooxygenase-2 (COX-2) inhibitors such as celecoxib, rofecoxib, and parecoxib; (h) inhibitors of phosphodiesterase type IV (PDE-IV); (i) 25 antagonists of the chemokine receptors, especially CCR-1, CCR-2, and CCR-3; (j) cholesterol lowering agents such as HMG-CoA reductase inhibitors (lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, and other statins), sequestrants (cholestyramine and colestipol), nicotinic acid, fenofibric acid derivatives (gemfibrozil, clofibrate, fenofibrate and benzofibrate), and probucol; (k) anti-diabetic agents such as insulin, sulfonylureas, biguanides (metformin), a-glucosidase inhibitors (acarbose) and 30 glitazones (troglitazone, pioglitazone, englitazone, MCC-555, BRL49653 and the like); (l) preparations of interferon beta (interferon beta-1a, interferon beta-1b); (m) anticholinergic agents such as muscarinic antagonists (ipratropium and tiotropium); (n) current treatments for multiple sclerosis, including prednisolone, glatiramer, deoxyadenosine, mitoxantrone, methotrexate, and cyclophosphamide; (o) p38 kinase inhibitors; (p) other compounds such as 5-aminosalicylic acid and prodrugs thereof, antimetabolites such as azathioprine and 6-mercaptopurine, and cytotoxic cancer chemotherapeutic 35 agents.

The weight ratio of the compound of the Formula I to the second active ingredient may be varied and will depend upon the effective dose of each ingredient. Generally, an effective dose of each will be used. Thus, for example, when a compound of the Formula I is combined with an NSAID the weight ratio of the compound of the Formula I to the NSAID will generally range from about 1000:1

to about 1:1000, preferably about 200:1 to about 1:200. Combinations of a compound of the Formula I and other active ingredients will generally also be within the aforementioned range, but in each case, an effective dose of each active ingredient should be used.

Abbreviations that may be used in the following Schemes and Examples include:

- 5 4-DMAP: 4-dimethylaminopyridine; AcCN: acetonitrile; BOC: tert-butoxycarbonyl; BOC-ON:2-(tert-butoxycarbonyloxyimino)-2-phenylacetonitrile; BOP: benzotriazol-1-yloxy-tris(dimethylamino)-phosphonium hexafluorophosphate; brine: saturated NaCl solution; DIPEA: N,N-diisopropylethylamine; DMF: dimethylformamide; DMSO: dimethylsulfoxide; Et: ethyl; EtOAc: ethyl acetate; EtOH: ethanol; g or gm: gram; h or hr: hours; HATU: *O*-(7-azabenzotriazol-1-yl)-*I*,*I*,*I*,*3*-tetramethyluronium hexafluorophosphate; HBTU: *O*-(benzotriazol-1-yl)-*I*,*I*,*I*,*3*-tetramethyluronium hexafluorophosphate; HOAc: acetic acid; HOAt: 1-hydroxy-7-azabenzotriazole; HOBt: 1-hydroxybenzotriazole; HPLC: high pressure liquid chromatography; *in vacuo*: rotovaporation; Me: methyl; MeOH: methanol; mg: milligram; MHz: megahertz; min: minutes; mL: milliliter; mmol: millimole; MS or ms: mass spectrum; MsCl: methane-sulfonyl chloride; Ph: phenyl; Ph₃P: triphenylphosphine; PyBOP: (benzotriazol-1-yloxy)tritypyrrolidino-phosphonium hexafluorophosphate; rt: room temperature; TEA: triethylamine; TFA: trifluoroacetic acid; THF: tetrahydrofuran.
- 10 Compounds of the present invention may be prepared by procedures illustrated in the accompanying schemes. In Scheme 1, a substituted pyridyl-4-carboxylic acid derivative A is treated with thionyl chloride to make the carboxylic acid chloride derivative which is then reacted with a 4-amino-
15 (L)-phenylalanine derivative to yield the amide B. The N-BOC-protecting group in B is removed with strong acid (TFA or HCl) to afford the free amine C.

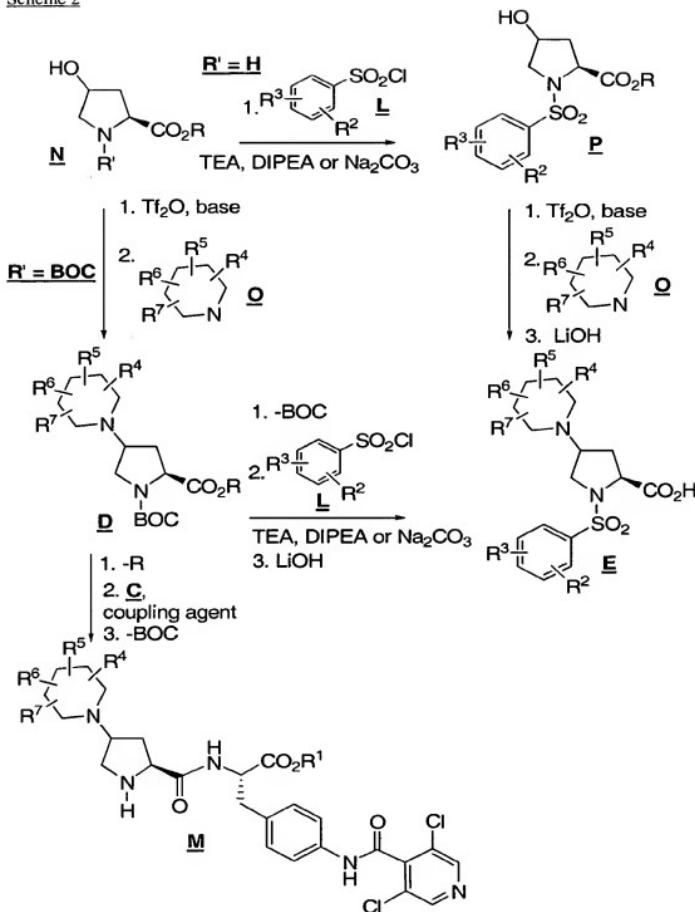
Scheme 1

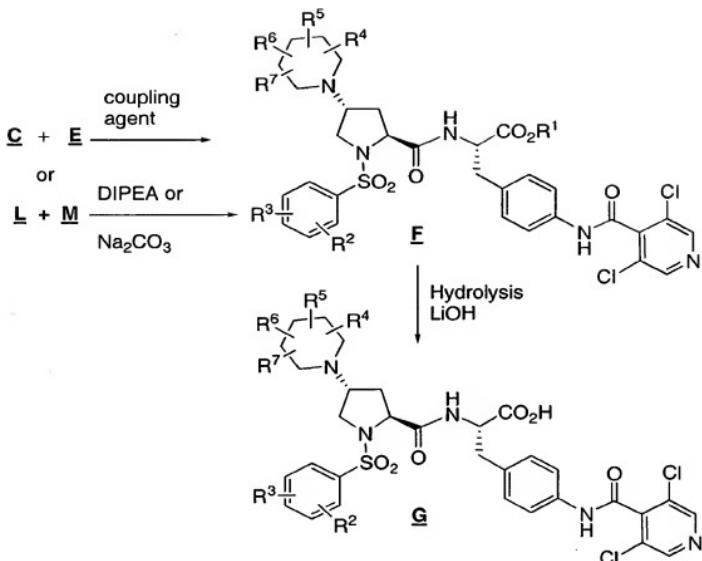
As shown in Scheme 2, an appropriately substituted (L)-proline ester **D** may be prepared by reacting the free alcohol of a suitably protected hydroxyproline N (R' = BOC) with an activating agent such as triflic anhydride in the presence of a suitable base such as DIPEA. To this intermediate, the desired amine derivative **O** is then added so furnishing derivative **D**. The BOC protecting group of **D** may then be removed using an acid such as TFA and then sulfonylated with a substituted benzene-10 sulfonyl chloride **L** in the presence of base (TEA, DIPEA or Na₂CO₃) to yield a sulfonamide which, upon treatment with hydroxide affords free acid **E**. Alternatively, sulfonamide **E** may be prepared from hydroxyproline **N** via intermediate **P**. Thus a suitably protected hydroxyproline derivative **N** (R' = H) maybe sulfonylated with a substituted benzenesulfonyl chloride **L** in the presence of base (TEA, DIPEA or Na₂CO₃) to yield sulfonamide **P**. Sulfonamide **P** may then be converted to **E** by reacting the free 15 alcohol with an activating agent such as triflic anhydride in the presence of a suitable base such as DIPEA. To this intermediate, the desired amine derivative **O** is then added. Finally, the ester protecting group is removed by treatment with hydroxide to afford the free acid **E**.

The proline ester **D** (R'=BOC) may alternatively be hydrolyzed to the corresponding acid by treatment with a base such as LiOH. The acid is then coupled with **C**, in the presence of an

appropriate coupling agent (e.g., PyBOP, HBTU/HOAt), to give **M**, following the removal of the BOC group. The final ester product **F** may be prepared when amine **C** and acid **E** are reacted together in the presence of an appropriate coupling agent (e.g., PyBOP, HBTU/HOAt, or **E** may be first converted to the corresponding acid chloride). Alternatively, the amine **M** is sulfonylated with **L** in the presence of a base 5 to provide **F** (Scheme 2). The ester in **F** can be hydrolyzed with hydroxide (if R¹ is n- or i-alkyl) or TFA or HCl (if R¹ is *tert*-butyl) to afford the corresponding acid **G**.

Purification of ester **F** or acid **G** may be carried out using normal or reverse phase chromatography. In the case of reverse phase chromatography, the presence of acid in the eluent (such as TFA or formic acid) may lead to the formation of the corresponding salt of **F** or **G**. If so desired, this salt 10 may be converted to the free base by treatment with a base such as sodium bicarbonate. Alternative salt forms of this free base may then be synthesized by treatment with the appropriate acid such as hydrochloric acid or methanesulfonic acid).

Scheme 2

Scheme 2 (con't)

5

Biological Evaluation

Compounds of formula I are potent antagonists of VLA-4 with significant and sustained receptor occupancy on VLA-4 bearing cells. The rate of dissociation of a test compound from VLA-4 on Jurkat cells may be determined by the method described in G. Doherty et al., *Bioorganic & Medicinal Chemistry Letters*, **13**, 1891 (2003).

- 10 Compounds of the present invention had half-lives of dissociation of greater than three hours ($t_{1/2} > 3$ hr) in this assay, demonstrating they are tight binding inhibitors of VLA-4.

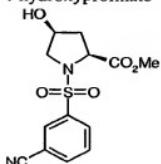
VLA-4 receptor occupancy after oral dosing in rats and dogs may be determined by the method described in D. R. Leone et al., *J. Pharmacol. Exper. Therap.*, **305**, 1150 (2003). Compounds of the present invention demonstrated sustained and significant receptor occupancy (>50%) after oral dosing.

Compounds of the present invention may be prepared by procedures detailed in the following examples. The examples provided are illustrative of the present invention and are not to be construed as limiting its scope in any manner:

5

REFERENCE EXAMPLE 1

Methyl (4S)-1-[(3-Cyanophenyl)sulfonyl]-4-hydroxyprolinate



Methyl (4S)-4-hydroxyprolinate (52.0 g, 0.286 mol) was dissolved in 500 mL CH_2Cl_2 and cooled to 0°C. Triethylamine (83.6 mL, 0.600 mol) was added, followed by 3-cyanobenzenesulfonyl chloride (55.0, 0.273 mol) slowly. The reaction mixture was stirred at room temperature overnight. Water was added, and the aqueous layer was extracted three times with CH_2Cl_2 . The combined organic layers were washed with 1N HCl, H_2O , 1N NaOH, and brine, dried over MgSO_4 , and concentrated to give a viscous residue. Ethyl acetate (300 mL) was added to completely dissolve the residue. Approximately 100 mL hexanes was added slowly until the solution became slightly cloudy. The mixture was then stirred overnight to allow the product to precipitate. The solid was filtered and rinsed two times with 30% EtOAc/hexanes to give the desired product as an off-white powder (66.2 g, 78%).
¹H-NMR (CDCl_3 , 500 MHz) δ 8.20-8.18 (m, 1H), 8.15-8.12 (m, 1H), 7.92-7.89 (m, 1H), 7.72 (t, 1H), 4.51-4.47 (m, 1H), 4.42 (br s, 1H), 3.74 (s, 3H), 3.53-3.49 (m, 1H), 3.46-3.43 (m, 1H), 3.39 (br s, 1H), 2.29-2.25 (m, 1H), 2.21-2.02 (m, 1H). MS (ESI) calculated for $\text{C}_{13}\text{H}_{14}\text{N}_2\text{O}_5\text{S}$ 310.3, observed m/e 20 311.2 (MH^+).

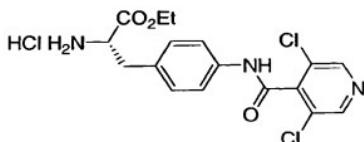
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REFERENCE EXAMPLE 2

4-(3',5'-Dichloroisonicotinoyl)amino)-(L)-phenylalanine, Ethyl Ester, HCl



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Step A: To 500 mL of absolute ethanol under nitrogen at 0°C was added thionyl chloride (21 mL, 0.29 mol) over 5 min, and the clear solution was stirred at 0°C for 10 min and then at rt for 30 min. 4-Nitro-L-phenylalanine (50.2 g, 0.24 mol) was added in one portion, and the mixture was refluxed overnight.

The resulting mixture was concentrated in *vacuo* to give 4-nitro-L-phenylalanine, ethyl ester, HCl (60 g) as a white solid. ¹H NMR (400 MHz, CD₃OD) δ 8.21 (d, 2H), 7.54 (d, 2H), 4.39 (dd, 1H), 4.22 (q, 2H), 3.24-3.40 (m, 2H), 1.22 (t, 3H).

Step B: To a suspension of the compound of Step A (60 g, 0.22 mol) in methylene chloride (1.5 L) under nitrogen was added TEA (31 mL). After stirring at rt for 10 min, di-*t*-butyl dicarbonate (49 g, 0.22 mol) and 4-DMAP (0.1 g) was added, and the reaction mixture was stirred at rt overnight, washed with 1N HCl (2x 200 mL), H₂O (2x 200 mL) and brine (1 X 250 mL), dried over anhydrous Na₂SO₄, filtered and concentrated to afford N-BOC-4-nitro-L-phenylalanine, ethyl ester (78 g). ¹H NMR (400 MHz, CDCl₃) δ 8.14 (d, 2H), 7.28 (d, 2H), 4.30-4.65 (m, 1H), 4.15 (q, 2H), 3.00-3.30 (m, 2H), 1.35 (s, 9H), 1.20 (t, 3H).

Step C: A solution of the compound of Step B (78.3 g, 0.22 mol) in absolute ethanol (300 mL) was purged with nitrogen, and 10% palladium on carbon (1.0 g) was added. After hydrogenated at 40-50 psi for 1 h, the reaction mixture was filtered through Celite, and the cake was washed with EtOH followed by EtOAc. The filtrate was concentrated, and the residue was purified by flash column chromatography on silica gel eluting with 4:1 to 1:1 EtOAc/Hexanes to afford N-BOC-4-amino-L-phenylalanine, ethyl ester (60 g). ¹H NMR (400 MHz, CDCl₃) δ 6.90 (d, 2H), 6.63 (d, 2H), 4.20-4.50 (m, 1H), 4.14 (q, 2H), 3.76-3.00 (m, 2H), 1.36 (s, 9H), 1.20 (t, 3H).

Step D: A nitrogen flushed 500 mL round bottom flask was charged with 3,5-dichloroisocinic acid (46.5 g, 0.24 mol), CH₂Cl₂ (150 mL), DMF (0.5 mL), and thionyl chloride (20 mL, 33.9 g 0.28 mol).

After the slurry was refluxed for 5 h, additional thionyl chloride (5 mL, 0.70 mol) and CH₂Cl₂ (100 mL) were added, and the reaction mixture was refluxed for additional 45 min and concentrated, and the residue was azeotroped with toluene to give the crude acyl chloride, which was used immediately. The crude acyl chloride was dissolved in CH₂Cl₂ (150 mL) and added to the compound of Step C (60 g, 0.20 mol) and 4-methylmorpholine (44 mL, 0.40 mol) in CH₂Cl₂ (400 mL) at 0°C over 5 min. After stirring at 0°C for 1 h, the reaction was quenched with dilute aqueous NaHCO₃. The organic layer was separated and the aqueous layer was extracted with CH₂Cl₂ (500 mL). The organic layers were combined, dried over anhydrous MgSO₄ and concentrated in *vacuo*, and the residue was purified by flash column chromatography on silica gel eluting with 4:1 to 3:2 EtOAc/hexanes to afford N-BOC-4-((3',5'-dichloroisocinoyl)amino)-L-phenylalanine, ethyl ester (95 g). ¹H NMR (400 MHz, CD₃OD) δ 8.60 (s, 2H),

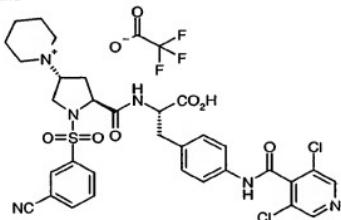
7.54 (d, 2H), 7.20 (d, 2H), 4.20-4.36 (m, 1H), 4.10 (q, 2H), 3.02-3.12 (m, 1H), 2.82-2.92 (m, 1H), 1.34/1.30 (s, 9H), 1.20 (t, 3H).

Step E: A solution of the compound of Step D (95 g, 0.197 mol) in EtOAc (1.2 L) was treated with a stream of hydrogen chloride gas over 2 h at rt. The resulting yellow suspension was diluted with hexanes (250 mL), cooled to 0°C and filtered. The cake was washed with hexanes and dried *in vacuo* to afford the title compound as a yellow solid (80 g). ¹H NMR (400 MHz, CD₃OD) δ 8.64 (s, 2H), 7.66 (d, 2H), 7.30 (d, 2H), 4.28 (dd, 1H), 4.25 (q, 2H), 3.20 (q, 2H), 1.26 (t, 3H).

10

EXAMPLE 1

(4*R*)-1-[(3-Cyanophenyl)sulfonyl]-4-(piperidinium-1-yl)-L-prolyl-4-{(3,5-dichloroisonicotinoyl)amino}-L-phenylalanine Trifluoroacetate



Step A: (4*R*)-1-[(3-cyanophenyl)sulfonyl]-4-(piperidin-1-yl)proline

15 Compound of Reference Example 1 (3.0 g, 9.67 mmol) was dissolved in 100 mL CH₂Cl₂. *N,N*-Diisopropylethylamine (4.23 mL, 24.22 mmol) was added and the reaction mixture was cooled to -78°C under N₂. Trifluoromethanesulfonic anhydride (2.28 mL, 13.55 mmol) was added dropwise to the solution over 10 minutes. The mixture was stirred for 1 hour at -78°C and then slowly warmed to -30°C over 30 minutes. Piperidine (1.91 mL, 19.31 mmol) was added dropwise over 5 minutes, and the solution was allowed to warm to room temperature and stirred overnight. 50 mL H₂O was added to the solution. The aqueous layer was extracted three times with CH₂Cl₂. The combined organic layers were dried over MgSO₄ and concentrated. The residue was purified by flash chromatography to give methyl (4*R*)-1-[(3-cyanophenyl)sulfonyl]-4-(piperidin-1-yl)proline (3.61 g, 99%) as a orange oil, which was dissolved in 15 mL acetonitrile and 5 mL H₂O. Lithium hydroxide 20 (1.00 g, 23.84 mmol) was added, and the reaction mixture was stirred at room temperature for 1 hour. LCMS showed no starting material remained, so the reaction mixture was neutralized with 23.84 mL 1N HCl (23.84 mmol). The solution was frozen in an acetone-dry ice bath and lyophilized for 16 hours to 25

give crude (*4R*)-1-[(3-cyanophenyl)sulfonyl]-4-(piperidin-1-yl)proline, which was used directly in the next reaction without further purification. MS (ESI) calculated for C₁₇H₂₁N₃O₄S 363.4, observed m/e 364.2 (M⁺).

5 **Step B:** Ethyl (*4R*)-1-[(3-Cyanophenyl)sulfonyl]-4-(piperidinium-1-yl)-L-prolyl-4-[(3,5-dichloro-isonicotinoyl)amino]-L-phenylalaninate Trifluoroacetate

The compound of Step A (9.56 mmol) was suspended in 25 mL DMF. The compound of Reference Example 2, (3.99 g, 9.53 mmol), HATU (3.99 g, 10.49 mmol), and N-methylmorpholine (2.62 mL, 23.83 mmol) were added, and the reaction mixture was stirred for 24 hours at room temperature.

10 The suspension was added dropwise to 100 mL H₂O with vigorous stirring. The precipitate was filtered immediately and washed three times with H₂O. The pale yellow powder was dried under vacuum and then purified by preparative reverse-phase HPLC to give the title compound (4.42 g, 55%) as a white fluffy solid. ¹H-NMR (MeOD, 500 MHz) δ 8.63 (s, 2H), 8.49 (d, 1H), 8.15 (s, 1H), 8.04-8.01 (m, 1H), 7.91-7.87 (m, 1H), 7.71 (t, 1H), 7.64 (d, 2H), 7.34 (d, 2H), 4.63-4.57 (m, 2H), 4.18 (q, 2H),
 15 3.96-3.93 (m, 2H), 3.49-3.44 (m, 3H), 3.25-3.20 (m, 1H), 3.06-2.97 (m, 3H), 2.40-2.37 (m, 1H), 2.20-2.16 (m, 1H), 1.98-1.93 (m, 2H), 1.80-1.78 (m, 1H), 1.70-1.66 (m, 2H), 1.54-1.50 (m, 1H), 1.25 (t, 3H). MS (ESI) calculated for C₃₄H₃₆Cl₂N₆O₆S 727.7, observed m/e 727.2 (M⁺).

20 **Step C:** (*4R*)-1-[(3-Cyanophenyl)sulfonyl]-4-piperidinium-1-yl-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalanine Trifluoroacetate

The compound of Step B (0.100 g, 0.14 mmol) was dissolved in 1.5 mL acetonitrile and 0.5 mL H₂O. Lithium hydroxide (0.014 g, 0.33 mmol) was added, and the reaction mixture was stirred at room temperature for 1 hour. LCMS showed no starting material remained, so the reaction mixture was neutralized with trifluoroacetic acid (0.03 mL, 0.33 mmol). The solution purified by preparative reverse-phase HPLC to give the title compound (0.043 g, 45%) as a fluffy white solid. ¹H-NMR (MeOD, 500 MHz) δ 8.63 (s, 2H), 8.45 (d, 1H), 8.13 (s, 1H), 8.01 (d, 1H), 7.82 (d, 1H), 7.69-7.61 (m, 3H), 7.36 (d, 2H), 4.65-4.57 (m, 2H), 3.98-3.88 (m, 3H), 3.50-3.44 (m, 3H), 3.31-3.25 (m, 1H), 3.04-2.98 (m, 2H), 2.47-2.41 (m, 1H), 2.23-2.15 (m, 1H), 2.12-1.44 (m, 7H). MS (ESI) calculated for C₃₂H₃₂Cl₂N₆O₆S 699.6, observed m/e 699.1 (M⁺).

30

EXAMPLE 1A

Ethyl (*4R*)-1-[(3-Cyanophenyl)sulfonyl]-4-(piperidinium-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalaninate, and HCl and methanesulfonic acid salts

The pale yellow crude powder of Example 1, Step B was purified by normal phase silica chromatography eluting with EtOAc (1% NH₄OH), followed by EtOAc (1% NH₄OH) / MeOH = 99/1 to afford the compound free base as a white solid.

The title compound free base (200 mg, 0.28 mmol) was dissolved in EtOAc.

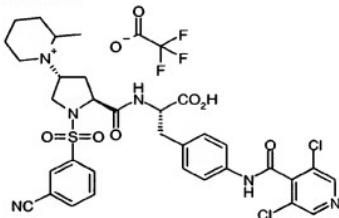
5 Hydrochloric acid (4N in dioxane, 100 μ L) was added dropwise and stirred at room temperature for 30 min. The precipitate obtained was filtered, washed with diethyl ether (3X) and dried under high vacuum to give the title compound HCl salt as a white solid.

The title compound free base (5.7g, 7.8 mmol) was dissolved in EtOH (200 mL).

Methanesulfonic acid (570 μ L) was added dropwise and diethyl ether (200 mL) was added. The reaction 10 mixture was stirred at room temperature for 4 hours. The precipitate obtained was filtered, washed with diethyl ether (3X) and dried under high vacuum to give the title compound methanesulfonic acid salt as a white solid.

EXAMPLE 2

15 1-[(3-Cyanophenyl)sulfonyl]-4-(2-methylpiperidinium-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalanine Trifluoroacetate



Starting with 2-methylpiperidine the general procedure of Example 1, Steps A and B was followed to yield ethyl 1-[(3-cyanophenyl)sulfonyl]-4-(2-methylpiperidinium-1-yl)-L-prolyl-4-[(3-

20 dichloroisonicotinoyl)amino]-L-phenylalaninate trifluoroacetate ¹H-NMR (MeOD, 500 MHz) δ 8.63 (s, 3H), 8.21-8.09 (m, 1H), 8.05-8.01 (m, 1H), 7.97-7.88 (m, 1H), 7.77-7.69 (m, 1H), 7.61 (d, 2H), 7.34 (d, 2H), 4.63 (d, 1H), 4.64-4.51 (m, 1H), 4.19-4.14 (m, 2H), 3.99-3.78 (m, 1H), 3.80-3.67 (m, 1H), 3.53-3.38 (m, 1H), 3.29-3.19 (m, 2H), 3.09-3.00 (m, 1H), 2.37-2.32 (m, 1H), 2.26-2.10 (m, 1H), 2.04-1.79 (m, 3H), 1.78-1.55 (m, 3H), 1.42-1.30 (m, 3H), 1.29-1.22 (m, 3H). MS (ESI) calculated for C₃₅H₃₈Cl₂N₆O₆S 25 741.7, observed m/e 741.2 (M+).

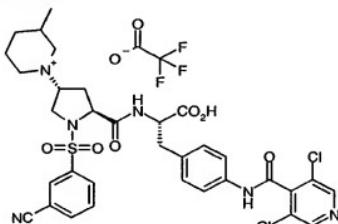
The title compound was prepared from the above ester following the procedure described for Example 1, Step C. ¹H-NMR (MeOD, 500 MHz) δ 8.63 (s, 2H), 8.18-8.12 (m, 1H), 8.04-7.99 (m,

1H), 7.93-7.78 (m, 1H), 7.74-7.65 (m, 3H), 7.37 (d, 2H), 4.68-4.53 (m, 2H), 3.99-3.88 (m, 2H), 3.78-3.67 (m, 1H), 3.54-3.38 (m, 2H), 3.31-3.29 (m, 1H), 3.28-3.14 (m, 2H), 3.09-2.99 (m, 1H), 2.51-2.38 (m, 1H), 2.04-1.56 (m, 7H), 1.42-1.30 (m, 3H). MS (ESI) calculated for C₃₃H₃₄Cl₂N₆O₆S 713.6, observed m/e 713.2 (M⁺).

5

EXAMPLE 3

1-[(3-Cyanophenyl)sulfonyl]-4-(3-methylpiperidinium-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalanine Trifluoroacetate



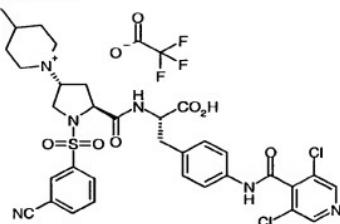
10 Starting with 3-methylpiperidine, the general procedure described in Example 1, Steps A and B was followed to provide ethyl 1-[(3-cyanophenyl)sulfonyl]-4-(3-methylpiperidinium-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)amino]-L-phenylalaninate trifluoroacetate. ¹H-NMR (MeOD, 500 MHz) δ 8.63 (s, 2H), 8.59-8.54 (m, 1H), 8.15 (d, 1H), 8.02 (d, 1H), 7.87 (t, 1H), 7.70 (dt, 1H), 7.65 (d, 2H), 7.34 (d, 2H), 4.64-4.56 (m, 2H), 4.18 (q, 2H), 3.97-3.91 (m, 2H), 3.55-3.43 (m, 2H), 3.42-3.35 (m, 2H), 3.27-3.20 (m, 1H), 3.08-3.00 (m, 1H), 2.97-2.83 (m, 1H), 2.71-2.58 (m, 1H), 2.49-2.39 (m, 1H), 2.31-2.20 (m, 1H), 2.01-1.92 (m, 1H), 1.89-1.80 (m, 2H), 1.78-1.68 (m, 1H), 1.25 (t, 3H), 1.22-1.13 (m, 1H), 0.98 (d, 3H). MS (ESI) calculated for C₃₅H₃₈Cl₂N₆O₆S 741.7, observed m/e 741.2 (M⁺).

15 The title compound was prepared from the above ester following the procedure described for Example 1, Step C. ¹H-NMR (MeOD, 500 MHz) δ 8.62 (s, 2H), 8.48-8.43 (m, 1H), 8.13 (d, 1H), 8.00 (d, 1H), 7.82 (t, 1H), 7.69-7.63 (m, 3H), 7.36 (d, 2H), 4.65-4.55 (m, 2H), 3.95-3.88 (m, 2H), 3.53-3.41 (m, 2H), 3.40-3.33 (m, 1H), 3.30-3.28 (m, 1H), 3.05-2.98 (m, 1H), 2.97-2.79 (m, 1H), 2.73-2.59 (m, 1H), 2.54-2.43 (m, 1H), 2.31-2.21 (m, 1H), 2.02-1.92 (m, 1H), 1.89-1.80 (m, 2H), 1.79-1.68 (m, 1H), 1.28-1.17 (m, 1H), 0.98 (d, 3H). MS (ESI) calculated for C₃₃H₃₄Cl₂N₆O₆S 713.6, observed m/e 713.2 (M⁺).

25

EXAMPLE 4

1-[(3-Cyanophenyl)sulfonyl]-4-(4-methylpiperidinium-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalanine Trifluoroacetate

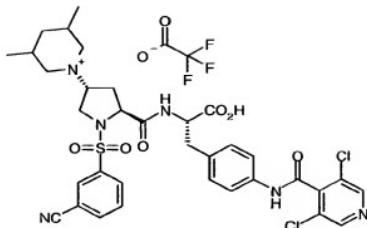


Starting with 4-methylpiperidine the general procedure described in Examples 1, Steps A and B was followed to provide ethyl 1-[(3-cyanophenyl)sulfonyl]-4-(4-methylpiperidinium-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)amino]-L-phenylalaninate trifluoroacetate. $^1\text{H-NMR}$ (MeOD, 500 MHz) δ 8.63 (s, 2H), 8.56 (d, 1H), 8.15 (s, 1H), 8.01 (d, 1H), 7.87 (d, 1H), 7.69 (t, 1H), 7.65 (d, 2H), 7.34 (d, 2H), 4.62-4.57 (m, 2H), 4.17 (q, 2H), 3.96-3.90 (m, 2H), 3.54-3.43 (m, 3H), 3.23 (dd, 1H), 3.09-2.92 (m, 3H), 2.43-2.39 (m, 1H), 2.28-2.20 (m, 1H), 1.92 (d, 2H), 1.77-1.63 (m, 1H), 1.43-1.31 (m, 2H), 1.25 (t, 3H), 0.98 (d, 3H). MS (ESI) calculated for $\text{C}_{35}\text{H}_{38}\text{Cl}_2\text{N}_6\text{O}_6\text{S}$ 741.7, observed m/e 743.2 (MH^+).

The title compound was prepared from the above ester following the procedure described for Example 1, Step C. $^1\text{H-NMR}$ (MeOD, 500 MHz) δ 8.63 (s, 2H), 8.45 (d, 1H), 8.13 (s, 1H), 8.00 (d, 1H), 7.83 (d, 1H), 7.69-7.63 (m, 3H), 7.36 (d, 2H), 4.64-4.56 (m, 2H), 3.93-3.88 (m, 2H), 3.50-3.43 (m, 3H), 3.40-3.25 (m, 1H), 3.09-2.93 (m, 3H), 2.46-2.41 (m, 1H), 2.24-2.17 (m, 1H), 1.92 (d, 2H), 1.79-1.63 (m, 1H), 1.45-1.30 (m, 2H), 0.98 (d, 3H). MS (ESI) calculated for $\text{C}_{33}\text{H}_{34}\text{Cl}_2\text{N}_6\text{O}_6\text{S}$ 713.6, observed m/e 713.1 (M^+).

EXAMPLE 5

1-[(3-Cyanophenyl)sulfonyl]-4-(3,5-dimethylpiperidinium-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalanine trifluoroacetate



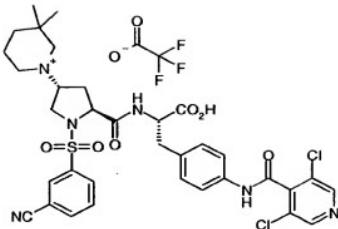
Starting with 3,5-dimethylpiperidine the general procedure of Example 1, Steps A and B was followed to provide ethyl 1-[(3-cyanophenyl)sulfonyl]-4-(3,5-dimethylpiperidinium-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)amino]-L-phenylalaninate trifluoroacetate. $^1\text{H-NMR}$ (MeOD, 500 MHz) δ

- 5 8.63 (s, 2H), 8.58 (d, 1H), 8.15 (s, 1H), 8.02 (d, 1H), 7.87 (d, 1H), 7.71 (t, 1H), 7.66 (d, 2H), 7.35 (d, 2H), 4.64-4.53 (m, 2H), 4.18 (q, 2H), 4.03-3.92 (m, 2H), 3.56-3.50 (m, 1H), 3.43-3.35 (m, 2H), 3.28-3.19 (m, 1H), 3.07-3.00 (m, 1H), 2.62-2.51 (m, 2H), 2.49-2.42 (m, 1H), 2.32-2.22 (m, 1H), 1.91-1.80 (m, 3H), 1.25 (t, 3H), 0.98 (d, 6H), 0.86 (q, 1H). MS (ESI) calculated for $\text{C}_{36}\text{H}_{40}\text{Cl}_2\text{N}_6\text{O}_6\text{S}$ 755.7, observed m/e 755.4 (M^+).

- 10 The title compound was prepared from the above ester following the procedure described for Example 1, Step C. $^1\text{H-NMR}$ (MeOD, 500 MHz) δ 8.62 (s, 2H), 8.47 (d, 1H), 8.13 (s, 1H), 8.00 (d, 1H), 7.82 (d, 1H), 7.69-7.63 (m, 3H), 7.37 (d, 2H), 4.64 (d, 1H), 4.61-4.56 (m, 1H), 4.02-3.91 (m, 2H), 3.51 (t, 1H), 3.41-3.34 (m, 2H), 3.31-3.27 (m, 1H), 3.06-2.99 (m, 1H), 2.62-2.48 (m, 3H), 2.29-2.21 (m, 1H), 1.92-1.81 (m, 3H), 0.98 (d, 6H), 0.86 (q, 1H). MS (ESI) calculated for $\text{C}_{34}\text{H}_{36}\text{Cl}_2\text{N}_6\text{O}_6\text{S}$ 727.6, observed m/e 727.2 (M^+).

EXAMPLE 6

1-[(3-Cyanophenyl)sulfonyl]-4-(3,3-dimethylpiperidinium-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalanine Trifluoroacetate



Starting with 3,3-dimethylpiperidine the general procedure of Example 1, Steps A and B was followed to provide ethyl 1-[{(3-cyanophenyl)sulfonyl]-4-(3,3-dimethylpiperidinium-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)amino]-L-phenylalaninate trifluoroacetate. $^1\text{H-NMR}$ (MeOD, 500 MHz) δ

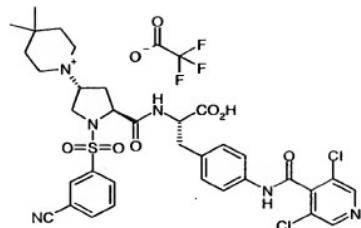
- 5 8.63 (s, 2H), 8.55-8.51 (m, 2H), 8.13 (d, 1H), 8.02 (t, 1H), 7.89-7.83 (m, 1H), 7.73-7.62 (m, 3H), 7.34 (d, 2H), 4.64-4.60 (m, 1H), 4.58-4.55 (m, 1H), 4.18 (q, 2H), 3.99-3.91 (m, 2H), 3.54-3.44 (m, 2H), 3.25-3.19 (m, 1H), 3.16 (d, 1H), 3.04-3.00 (m, 1H), 2.97-2.79 (m, 2H), 2.53-2.21 (m, 2H), 1.89-1.82 (m, 2H), 1.58-1.53 (m, 1H), 1.48-1.41 (m, 1H), 1.24 (t, 3H), 1.09 (d, 3H), 1.03 (s, 3H). MS (ESI) calculated for $\text{C}_{36}\text{H}_{40}\text{Cl}_2\text{N}_2\text{O}_6\text{S}$ 755.7, observed m/e 757.1 (MH $^+$).

- 10 The title compound was prepared from the above ester following the procedure described for Example 1, Step C. $^1\text{H-NMR}$ (MeOD, 500 MHz) δ 8.62 (s, 2H), 8.48 (d, 1H), 8.12 (s, 1H), 8.00 (d, 1H), 7.83-7.73 (m, 1H), 7.69-7.64 (m, 3H), 7.37 (d, 2H), 4.64 (d, 1H), 4.60-4.55 (m, 1H), 3.99-3.87 (m, 2H), 3.53-3.41 (m, 2H), 3.31-3.25 (m, 1H), 3.19-3.11 (m, 1H), 3.04-2.97 (m, 1H), 2.96-2.81 (m, 2H), 2.57-2.21 (m, 2H), 1.87-1.82 (m, 2H), 1.64-1.38 (m, 2H), 1.05 (s, 6H). MS (ESI) calculated for

- 15 $\text{C}_{34}\text{H}_{36}\text{Cl}_2\text{N}_2\text{O}_6\text{S}$ 727.6, observed m/e 727.2 (M $^+$).

EXAMPLE 7

1-[(3-Cyanophenyl)sulfonyl]-4-(4,4-dimethylpiperidinium-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalanine Trifluoroacetate



Starting with 4,4-dimethylpiperidine the general procedure of Example 1, Steps A and B was followed to provide ethyl 1-[(3-cyanophenyl)sulfonyl]-4-(4,4-dimethylpiperidinium-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)amino]-L-phenylalaninate trifluoroacetate. ¹H-NMR (MeOD, 500 MHz) δ

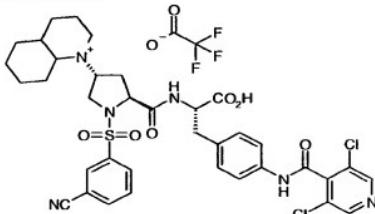
- 5 8.63 (s, 2H), 8.58 (d, 1H), 8.15 (s, 1H), 8.01 (d, 1H), 7.87 (d, 1H), 7.69 (t, 1H), 7.65 (d, 2H), 7.34 (d, 2H), 4.64-4.57 (m, 2H), 4.18 (q, 2H), 4.10-4.03 (m, 1H), 3.94 (t, 1H), 3.49 (t, 1H), 3.42-3.33 (m, 2H), 3.27-3.20 (m, 1H), 3.19-3.09 (m, 2H), 3.08-3.01 (m, 1H), 2.45-2.40 (m, 1H), 2.30-2.21 (m, 1H), 1.69-1.62 (m, 4H), 1.24 (t, 3H), 1.04 (br s, 6H). MS (ESI) calculated for C₃₆H₄₀Cl₂N₆O₆S 755.7, observed m/e 757.1 (MH⁺).

10 The title compound was prepared from the above ester following the procedure described for Example 1, Step C. ¹H-NMR (MeOD, 500 MHz) δ 8.62 (s, 2H), 8.47 (d, 1H), 8.13 (s, 1H), 8.01 (d, 1H), 7.83 (d, 1H), 7.69-7.63 (m, 3H), 7.37 (d, 2H), 4.64-4.57 (m, 2H), 4.07-4.02 (m, 1H), 3.93 (t, 1H), 3.49 (t, 1H), 3.41-3.31 (m, 2H), 3.30-3.25 (m, 1H), 3.24-3.09 (m, 2H), 3.05-2.98 (m, 1H), 2.48-2.42 (m, 1H), 2.29-2.19 (m, 1H), 1.70-1.59 (m, 4H), 1.03 (s, 6H). MS (ESI) calculated for C₃₄H₃₆Cl₂N₆O₆S

15 727.6, observed m/e 727.2 (M⁺).

EXAMPLE 8

1-[(3-Cyanophenyl)sulfonyl]-4-(octahydroquinolinium-1(2*H*)-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalanine Trifluoroacetate



20

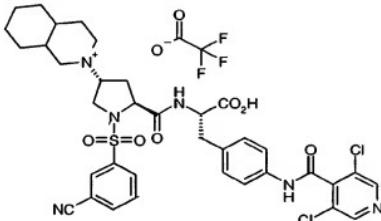
Starting with decahydroquinoline the general procedure of Example 1, Steps A and B was followed to provide ethyl 1-[(3-cyanophenyl)sulfonyl]-4-(octahydroquinolinium-1(2*H*)-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)amino]-L-phenylalaninate trifluoroacetate. ¹H-NMR (MeOD, 500 MHz) δ 8.65-8.61 (m, 2H), 8.59-8.52 (m, 1H), 8.23-8.18 (m, 1H), 8.08-8.01 (m, 1H), 8.01-7.89 (m, 1H), 7.78-7.68 (m, 1H), 7.67-7.57 (m, 2H), 7.38-7.23 (m, 2H), 4.71-4.50 (m, 2H), 4.24-4.13 (m, 2H), 4.06-3.92 (m, 2H), 3.59-3.48 (m, 1H), 3.45-3.39 (m, 1H), 3.29-3.14 (m, 3H), 3.12-3.01 (m, 2H), 2.47-2.30 (m, 1H), 2.22-2.12 (m, 1H), 2.11-2.02 (m, 1H), 2.01-1.58 (m, 8H), 1.52-1.32 (m, 4H), 1.30-1.21 (m, 3H). MS (ESI) calculated for C₃₈H₄₂Cl₂N₆O₆S 781.7, observed m/e 781.4 (M⁺).

The title compound was prepared from the above ester following the procedure described for Example 1, Step C. ¹H-NMR (MeOD, 500 MHz) δ 8.64-8.60 (m, 2H), 8.52-8.40 (m, 1H), 8.22-8.16 (m, 1H), 8.05-8.00 (m, 1H), 7.99-7.86 (m, 1H), 7.75-7.63 (m, 3H), 7.41-7.35 (m, 2H), 4.67-4.49 (m, 2H), 4.02-3.90 (m, 2H), 3.58-3.47 (m, 1H), 3.46-3.38 (m, 1H), 3.32-3.27 (m, 1H), 3.22-3.14 (m, 2H), 3.12-2.97 (m, 2H), 2.50-2.34 (m, 1H), 2.24-2.03 (m, 2H), 2.02-1.88 (m, 4H), 1.87-1.78 (m, 1H), 1.77-1.61 (m, 3H), 1.54-1.33 (m, 4H). MS (ESI) calculated for C₃₆H₃₉Cl₂N₆O₆S 753.7, observed m/e 753.3 (M⁺).

15

EXAMPLE 9

1-[(3-Cyanophenyl)sulfonyl]-4-(octahydroisoquinolinium-2(1*H*)-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)amino]-L-phenylalanine Trifluoroacetate

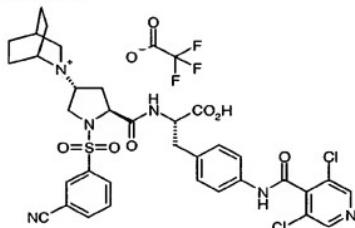


Starting with decahydroisoquinoline the general procedure of Example 1, Steps A and B was followed to provide ethyl 1-[(3-cyanophenyl)sulfonyl]-4-(octahydroisoquinolinium-2(1*H*)-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)amino]-L-phenylalaninate trifluoroacetate. ¹H-NMR (MeOD, 500 MHz) δ 8.63 (s, 2H), 8.61-8.54 (m, 1H), 8.16-8.13 (m, 1H), 8.03-8.00 (m, 1H), 7.88-7.83 (m, 1H), 7.73-7.67 (m, 1H), 7.63 (d, 2H), 7.34 (d, 2H), 4.66-4.54 (m, 2H), 4.19 (q, 2H), 4.16-3.91 (m, 2H), 3.57-3.46 (m, 1H), 3.32-3.03 (m, 5H), 2.54-2.07 (m, 3H), 2.06-1.70 (m, 5H), 1.69-1.58 (m, 3H), 1.55-1.30 (m, 5H), 1.25 (t, 3H). MS (ESI) calculated for C₃₈H₄₂Cl₂N₆O₆S 781.7, observed m/e 781.4 (M⁺).

The title compound was prepared from the above ester following the procedure described for Example 1, Step C. $^1\text{H-NMR}$ (MeOD , 500 MHz) δ 8.63 (s, 2H), 8.49-8.44 (m, 1H), 8.14-8.12 (m, 1H), 8.02-7.99 (m, 1H), 7.84-7.79 (m, 1H), 7.69-7.53 (m, 3H), 7.37 (d, 2H), 4.67-4.56 (m, 2H), 4.11-3.90 (m, 2H), 3.56-3.46 (m, 1H), 3.34-3.31 (m, 2H), 3.22-3.00 (m, 3H), 2.60-2.49 (m, 1H), 2.29-2.16 (m, 1H), 5 2.15-1.83 (m, 4H), 1.82-1.70 (m, 3H), 1.69-1.58 (m, 3H), 1.57-1.44 (m, 1H), 1.43-1.22 (m, 4H). MS (ESI) calculated for $\text{C}_{36}\text{H}_{39}\text{Cl}_2\text{N}_6\text{O}_6\text{S}$ 753.7, observed m/e 753.3 (M^+).

EXAMPLE 10

10 4-(2-Azoniabicyclo[2.2.2]oct-2-yl)-1-[(3-cyanophenyl)sulfonyl]-L-prolyl-4-[(3,5-dichloroisocinotinoyl)-amino]-L-phenylalanine Trifluoroacetate

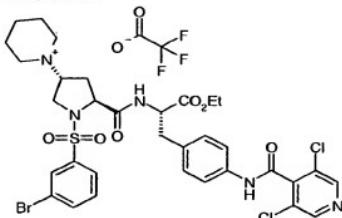


Starting with 2-azabicyclo[2.2.2]octane and using (2S)-3-{4-[(3,5-dichloroisocinotinoyl)-amino]phenyl}-1-methoxy-1-oxopropan-2-aminium chloride, the general procedure of Example 1, Steps A and B was followed to provide methyl (4*R*)-4-(2-azabicyclo[2.2.2]oct-2-yl)-1-[(3-cyanophenyl)sulfonyl]-L-prolyl-4-[(3,5-dichloroisocinotinoyl)amino]-D-phenylalaninate. $^1\text{H-NMR}$ (Acetone-d₆, 500 MHz) δ 9.92 (s, 1H), 8.67 (s, 2H), 8.23 (s, 1H), 8.11-8.06 (m, 2H), 7.83-7.75 (m, 4H), 7.53-7.45 (d, 2H), 4.78-4.76 (m, 1H), 4.62-4.60 (m, 1H), 4.22-4.15 (m, 2H), 3.64-3.58 (m, 3H), 3.42-3.37 (m, 3H), 3.30 (s, 3H), 3.25-3.21 (dd, 1H), 2.36-2.32 (m, 1H), 2.15-2.11 (m, 2H), 1.97 (m, 1H), 1.86-1.70 (m, 6H). MS (ESI) calculated for $\text{C}_{35}\text{H}_{36}\text{Cl}_2\text{N}_6\text{O}_6\text{S}$ 739.7, observed m/e 739.7 (M^+).

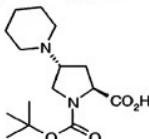
20 The title compound was prepared from the above ester following the procedure described for Example 1, Step C. $^1\text{H-NMR}$ (Acetone-d₆, 500 MHz) δ 9.93 (s, 1H), 8.66 (s, 2H), 8.23 (s, 1H), 8.11-8.06 (m, 2H), 7.80-7.71 (m, 4H), 7.41-7.39 (d, 2H), 4.78-4.76 (m, 1H), 4.62-4.60 (m, 1H), 4.22-4.20 (m, 1H), 4.03-4.00 (m, 1H), 3.64-3.58 (m, 3H), 3.40-3.38 (m, 2H), 3.27-3.24 (dd, 1H), 3.16-3.12 (dd, 1H), 2.36-2.32 (m, 1H), 2.18-2.14 (m, 2H), 1.97 (m, 1H), 1.86-1.70 (m, 5H), 1.29-1.31 (m, 1H). MS (ESI) calculated for $\text{C}_{34}\text{H}_{34}\text{Cl}_2\text{N}_6\text{O}_6\text{S}$ 725.7, observed m/e 725.7 (M^+).

EXAMPLE 11

Ethyl (4*R*)-1-[*(3*-Bromophenyl)sulfonyl]-4-piperidinium-1-yl-L-prolyl-4-[*(3,5*-dichloroisocotinoyl)-amino]-L-phenylalaninate Trifluoroacetate



5 Step A: (4*R*)-1-(*tert*-butoxycarbonyl)-4-piperidin-1-ylproline



1-*tert*-Butyl 2-methyl 4-hydroxypyrrolidine-1,2-dicarboxylate (3.0 g, 9.67 mmol) was dissolved in 50 mL CH₂Cl₂. *N,N*-Diisopropylethylamine (2.7 mL, 15.25 mmol) was added and the reaction mixture was cooled to -78°C under N₂. Trifluoromethanesulfonic anhydride (1.4 mL, 8.5 mmol) was added dropwise to the solution over 10 minutes. The mixture was stirred for 1 hour at -78°C and then slowly warmed to -30°C over 30 minutes. Piperidine (1.8 mL, 18.3 mmol) was added dropwise over 5 minutes, and the solution was allowed to warm to room temperature and stirred overnight. 25 mL H₂O was added to the solution. The aqueous layer was extracted three times with CH₂Cl₂. The combined organic layers were dried over MgSO₄ and concentrated. The residue was purified by flash chromatography to give methyl (4*R*)-1-(*tert*-butoxycarbonyl)-4-piperidin-1-ylproline (1.8 g, 95%) as an oil. ¹H-NMR (CDCl₃, 500 MHz) δ 4.44-4.31 (m, 1H), 3.87-3.75 (m, 1H), 3.72 (s, 3H), 3.45-2.90 (m, 3H), 2.51-2.15 (m, 5H), 1.71-1.55 (m, 4H), 1.48-1.39 (m, 11H). MS (ESI) calculated for C₁₆H₂₈N₂O₄ 312.4, observed m/e 313.3 (MH⁺).

The above methyl ester (0.73g, 2.3 mmol) was dissolved in 6 mL acetonitrile and 3 mL H₂O. Lithium hydroxide (0.29 g, 6.9 mmol) was added, and the reaction mixture was stirred at room temperature for 2.5 hours. LCMS showed no starting material remained, so the reaction mixture was neutralized with 7 mL 1N HCl (7 mmol). The solution was frozen in an acetone-dry ice bath and

lyophilized overnight to give the crude acid, which was used directly in the next reaction without further purification. MS (ESI) calculated for C₁₅H₂₆N₂O₄ 298.4, observed m/e 299.2 (M⁺).

Step B: Ethyl (4*R*)-1-(*tert*-Butoxycarbonyl)-4-piperidin-1-yl-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalaninate

The compound of Step A (2.3 mmol) was suspended in 10 mL DMF. (2S)-3-[4-[(3,5-dichloroisonicotinoyl)-amino]phenyl]-1-ethoxy-1-oxopropan-2-aminium chloride (1.1 g, 2.5 mmol), HATU (1.05 g, 2.8 mmol), and N-methylmorpholine (1.2 mL, 11 mmol) were added, and the reaction mixture was stirred for 24 hours at room temperature. The suspension was added dropwise to 50 mL H₂O with vigorous stirring. The precipitate was filtered immediately and washed three times with H₂O. The pale yellow powder was dried under vacuum and used directly in the next step. MS (ESI) calculated for C₃₂H₄₁Cl₂N₅O₆ 662.6, observed m/e 662.4 (M⁺).

Step C: Ethyl (4*R*)-1-[(3-Bromophenyl)sulfonyl]-4-piperidinium-1-yl-L-prolyl-4-[(3,5-dichloroisonicotinoyl)amino]-L-phenylalaninate Trifluoroacetate

The compound of Step B (900 mg, 1.36 mmol) was stirred in 20 mL of a CH₂Cl₂/TFA (3/1) solution for 1.5 hours. The solution was concentrated and the excess TFA was co-evaporated with toluene (3x20 mL). The crude material was dissolved in 20 mL of CH₂Cl₂ and the solution was cooled to 0°C. Triethylamine (2 mL, 14.3 mmol) and 3-bromophenylsulfonyl chloride (200 uL, 1.38 mmol) were added and the solution was stirred for 20 hours at room temperature. The solution was concentrated, dissolved in 10 mL of MeOH and purified by preparative reverse-phase HPLC to give the title compound as a powder. ¹H-NMR (MeOD, 500 MHz) δ 8.65 (s, 2H), 8.51 (d, 1H), 8.01 (t, 1H), 7.83-7.85 (m, 1H), 7.67-7.69 (m, 1H), 7.63 (d, 2H), 7.47 (t, 1H), 7.33 (d, 1H), 4.55-4.63 (m, 2H), 4.17 (q, 2H), 3.97-3.98 (m, 2H), 3.41-3.44 (m, 3H), 2.92-3.08 (m, 3H), 2.37-2.41 (m, 1H), 2.14-2.17 (m, 1H), 1.47-1.95 (m, 6H), 1.25 (t, 3H). MS (ESI) calculated for C₃₃H₃₆BrCl₂N₅O₆S 781.6, observed m/e 782.3 (M⁺).

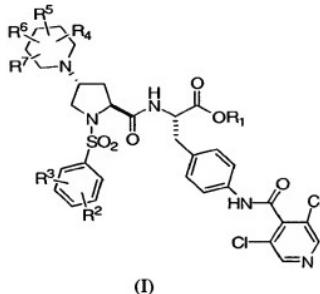
EXAMPLE 12

Ethyl (4*R*)-1-[(3,5-dichlorophenyl)sulfonyl]-4-piperidinium-1-yl-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalaninate Trifluoroacetate and (4*R*)-1-[(3,5-dichlorophenyl)sulfonyl]-4-piperidinium-1-yl-L-prolyl-4-[(3,5-dichloroisonicotinoyl)amino]-L-phenylalanine Trifluoroacetate

The title compounds may be prepared according to the procedures described in the previous examples.

WHAT IS CLAIMED IS:

1. A compound of formula I, or a pharmaceutically acceptable salt thereof, or pyridine-N-oxide thereof:



5

wherein

R¹ is selected from (1) hydrogen, (2) C₁-10alkyl, (3) -(C₁-10alkyl)-aryl, (4) -(C₁-10alkyl)-O-C₁-10alkyl, (5) -(C₁-10alkyl)-OC(O)-C₁-10alkyl, (6) -(C₁-10alkyl)-OC(O)-aryl, (7) -(C₁-10alkyl)-OC(O)O-

10 C₁-10alkyl, and (8) -(C₁-10alkyl)-N+(C₁-3alkyl)3;

R² and R³ are independently selected from H, -SO₂-C₁-3alkyl, CN, CF₃, OCF₃, and halogen;

R⁴, R⁵, R⁶ and R⁷ are independently selected from hydrogen and C₁-10alkyl; or

two of R⁴, R⁵, R⁶ and R⁷ together with the carbon atom(s) to which they are attached complete a 3-8 membered carbocyclic ring.

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2. A compound of Claim 1 wherein R¹ is hydrogen, C₁-4alkyl, -(C₁-4alkyl)OC₁-4alkyl, or -(C₁-4alkyl)N⁺(C₁-3alkyl)3.

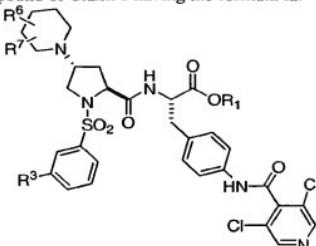
20 3. A compound of Claim 1 wherein R² is hydrogen and R³ is selected from -SO₂-C₁-3alkyl, CN and halogen.

4. A compound of Claim 1 wherein R² is hydrogen and R³ is CN.

25 5. A compound of Claim 1 wherein R⁴ and R⁵ are each hydrogen, and R⁶ and R⁷ are independently hydrogen or C₁-4alkyl.

6. A compound of Claim 1 wherein R⁴, R⁵, R⁶ and R⁷ are each hydrogen.

7. A compound of Claim 1 having the formula Ia:



Ia

5 or a pharmaceutically acceptable salt thereof, or pyridine N-oxide thereof, wherein

R¹ is selected from hydrogen, C₁-10alkyl, -(C₁-4alkyl)-aryl, -(C₁-4alkyl)-O-C₁-4alkyl, and -(C₁-4alkyl)-N⁺(C₁-3alkyl)3;

R³ is CN, halogen or -SO₂-C₁-3alkyl; and

10 R⁶ and R⁷ are independently hydrogen or C₁-4alkyl.

8. A compound of Claim 7 wherein R³ is CN.

9. A compound of Claim 7 wherein R⁶ and R⁷ are each hydrogen.

15

10. A compound selected from:

(4R)-1-[(3-cyanophenyl)sulfonyl]-4-(piperidin-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)amino]-L-phenylalanine;

(4R)-1-[(3-cyanophenyl)sulfonyl]-4-(2-methylpiperidin-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)amino]-L-phenylalanine;

(4R)-1-[(3-cyanophenyl)sulfonyl]-4-(3-methylpiperidin-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)amino]-L-phenylalanine;

(4R)-1-[(3-cyanophenyl)sulfonyl]-4-(4-methylpiperidin-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)amino]-L-phenylalanine;

25 (4R)-1-[(3-cyanophenyl)sulfonyl]-4-(3,5-dimethylpiperidin-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)amino]-L-phenylalanine;

(4*R*)-1-[(3-cyanophenyl)sulfonyl]-4-(3,3-dimethylpiperidin-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalanine;

(4*R*)-1-[(3-cyanophenyl)sulfonyl]-4-(4,4-dimethylpiperidin-1-yl)-L-prolyl-4-[(3,5-dichloroisonicotinoyl)-amino]-L-phenylalanine;

5 (4*R*)-1-[(3-cyanophenyl)sulfonyl]-4-(octahydroquinolin-1(2*H*)-yl)-L-prolyl-4-[(3,5-dichloro-isonicotinoyl)amino]-L-phenylalanine;

(4*R*)-1-[(3-cyanophenyl)sulfonyl]-4-(octahydroisoquinolin-2(1*H*)-yl)-L-prolyl-4-[(3,5-dichloro-isonicotinoyl)amino]-L-phenylalanine;

10 (4*R*)-4-(2-azabicyclo[2.2.2]oct-2-yl)-1-[(3-cyanophenyl)sulfonyl]-L-prolyl-4-[(3,5-dichloro-isonicotinoyl)amino]-L-phenylalanine;

Ethyl (4*R*)-1-[(3-bromophenyl)sulfonyl]-4-piperidin-1-yl-L-prolyl-4-[(3,5-dichloroisonicotinoyl)amino]-L-phenylalaninate;

and pharmaceutically acceptable salts thereof.

15 11. A pharmaceutical composition comprising a therapeutically effective amount of a compound of Claim 1 or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier.

20 12. Use of a compound of Claim 1 or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment or prevention of diseases mediated by cell adhesion.

13. The use of Claim 9 wherein said disease is selected from asthma, multiple sclerosis, inflammatory bowel disease, chronic obstructory pulmonary disease, sickle cell anemia, leukemia, and rheumatoid arthritis.

ABSTRACT OF THE DISCLOSURE

Compounds of Formula I are antagonists of VLA-4, and as such are useful in the inhibition or prevention of cell adhesion and cell-adhesion mediated pathologies. These compounds may be formulated into pharmaceutical compositions and are suitable for use in the treatment of inflammatory bowel disease including ulcerative colitis and Crohn's disease, multiple sclerosis, asthma, and rheumatoid arthritis.